Sofia®

RSV FIA

For use with the Sofia Analyzer only

INTENDED USE

The Sofia RSV FIA employs immunofluorescence for detection of respiratory syncytial virus (RSV) nucleoprotein antigen in nasopharyngeal swab and nasopharyngeal aspirate/wash specimens taken directly from symptomatic patients. This qualitative test is intended for use as an aid in the rapid diagnosis of acute RSV infections in pediatric patients less than 19 years of age. Negative results do not preclude RSV infection and should not be used as the sole basis for treatment or for other management decisions. A negative result is presumptive, and it is recommended these results be confirmed by virus culture or an FDA-cleared RSV molecular assay.

SUMMARY AND EXPLANATION

RSV is a causative agent of highly contagious, acute, viral infection of the respiratory tract in pediatric and elderly populations. Respiratory syncytial virus is a single-stranded RNA virus.[1] Nearly half of all children become infected by RSV in their first year of life. It is also the major viral cause of nosocomial illness in children already hospitalized for other reasons.[2] In the United States, RSV is estimated to be responsible for 73,400 to 126,300 hospitalizations annually for bronchiolitis and pneumonia alone among children younger than 1 year.[3] In an analysis of U.S. viral surveillance and mortality data, respiratory syncytial virus (RSV) was reported as the most common viral cause of death in children younger than 5 years when compared to influenza A (H1N1), influenza A (H3N2), and influenza B.[4] Among children hospitalized with RSV infection, the mortality rate is estimated to be as low as 0.3% to 1.0%[3, 5] and in the range of 2.5% to 4.0% for children with underlying cardiac or pulmonary disease.[3, 5, 6]

PRINCIPLE OF THE TEST

The Sofia RSV FIA test employs immunofluorescence technology that is used with the Sofia Analyzer for the rapid detection of RSV antigens. The Sofia RSV FIA test involves the disruption of RSV viral antigens. The patient specimen is placed in the Reagent Tube, during which time the virus particles in the specimen are disrupted, exposing internal viral nucleoproteins. After disruption, the specimen is dispensed into the Cassette sample well. From the sample well, the specimen migrates through a test strip containing various unique chemical environments. If RSV viral antigens are present, they will be trapped in a specific location.

Note: Depending upon the user's choice, the cassette is either placed inside of the Sofia Analyzer for automatically timed development (Walk Away Mode) or placed on the counter or bench top for a manually timed development and then placed into the Sofia Analyzer to be scanned (Read Now Mode).

The Sofia Analyzer will scan the test strip and measure the fluorescent signal by processing the results using method-specific algorithms. The Sofia Analyzer will display the test results (Positive, Negative, or Invalid) on the screen. The results can also be automatically printed on an integrated printer if this option is selected.

REAGENTS AND MATERIALS SUPPLIED

25 -Test Kit:

- Individually Packaged Cassettes (25): Mouse monoclonal anti-RSV antibodies
- Reagent Tubes (25): Lyophilized buffer with detergents and reducing agents
- Reagent Solution (25): Vials with salt solution
- Sterile Nasopharyngeal Swabs (25)
- Large, Pink Fixed Volume Pipettes (25)
- Small, Clear Fixed Volume Pipettes (25)
- RSV Positive Control Swab (1): Swab is coated with non-infectious RSV antigen
- Negative Control Swab (1): Swab is coated with heat-inactivated, non-infectious Streptococcus C antigen
- Package Insert (1)
- Quick Reference Instructions (1)
- QC Card (located on kit box)
- Printer Paper (1)

MATERIALS NOT SUPPLIED IN KIT

- Timer or watch for use in Read-Now Mode
- Sofia Analyzer instrument
- Sterile saline for the collection of Nasopharyngeal Aspirate or Wash Specimens
- Equipment used for collection of Nasopharyngeal Aspirate or Wash Specimens
- Calibration Cassette (supplied with the Sofia Analyzer)

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use.
- Do not use the kit contents beyond the expiration date printed on the outside of the box.
- Use appropriate precautions in the collection, handling, storage, and disposal of patient samples and used kit contents.[7]
- Use of Nitrile or Latex (or equivalent) gloves is recommended when handling patient samples.[7]
- Dispose of containers and used contents in accordance with Federal, State and Local requirements.
- Do not reuse the used cassette, fixed volume pipettes, reagent tubes, solutions, or control swabs.
- The user should never open the foil pouch of the test Cassette exposing it to the ambient environment until the Cassette is ready for immediate use.
- Discard and do not use any damaged cassette or material.
- The Reagent Solution contains a salt solution (saline). If the solution contacts the skin or eye, flush with copious amounts of water.
- To obtain accurate results, the Package Insert instructions must be followed.
- The Calibration Cassette must be kept in the provided storage pouch between uses.
- Inadequate or inappropriate specimen collection, storage, and transport may yield false test results.
- Specimen collection and handling procedures require specific training and guidance.
- Use the Viral Transport Media recommended in this Package Insert.
- When collecting a nasopharyngeal swab specimen, use the nasopharyngeal swab supplied in the kit.

- Use the appropriate fixed volume pipette in accordance with test procedures:
  - ► The **small, clear** fixed volume pipette is to be used ONLY for adding patient sample to the test cassette.
  - ► The **large, pink** fixed volume pipette is to be used ONLY with the aspirate/wash or viral transport media test procedure when transferring the patient sample from the collection cup into the Reagent Tube.
- Do not write on the barcode of the Cassette. This is used by the Sofia Analyzer to identify the type of test being run and to identify the individual Cassette so as to prevent a second read of the Cassette by the same Sofia Analyzer.
- Do not attempt to scan a Cassette more than one time. The barcode on the Cassette contains a unique identifier that will prevent the Sofia Analyzer from performing a second read on a previously scanned Cassette. An error message will be displayed if a Cassette is scanned more than once.
- As the detection reagent is a fluorescent compound, no visible results will form on the test strip. The Sofia Analyzer must be used for result interpretation.

KIT STORAGE AND STABILITY

Store the kit at room temperature, 59°F to 86°F (15°C to 30°C), out of direct sunlight. Kit contents are stable until the expiration date printed on the outer box. Do not freeze.

QUALITY CONTROL

There are three types of Quality Control for the Sofia Analyzer and Cassette: Sofia Analyzer Calibration Check procedure, built-in procedural control features, and External Controls.

Sofia Analyzer Calibration Check Procedure

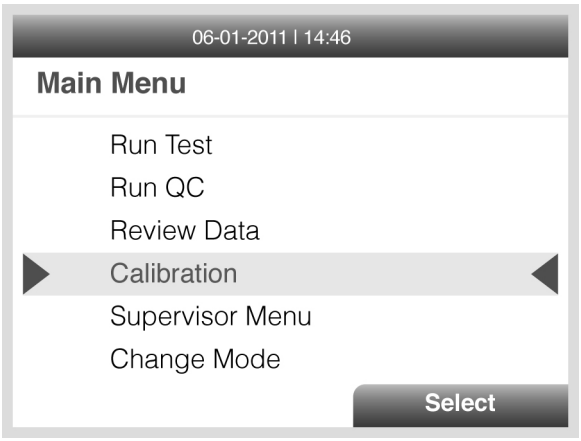
Note: This is a "Calibration Check" procedure.

The Calibration Check Procedure should be performed every thirty (30) days. The Sofia Analyzer can be set to remind the user to complete the Calibration Check Procedure.

The Calibration Check is a required function that checks the Sofia Analyzer optics and calculation systems using a specific Calibration Cassette. This Calibration Cassette is shipped with the Sofia Installation Pack. Refer to the Sofia Analyzer User Manual for details regarding the Calibration Check Procedure.

Important: Ensure that the Calibration Cassette is stored in the provided storage pouch between uses to protect from exposure to light.

1. To check the calibration of the Sofia Analyzer, select "Calibration" from the Main Menu.



2. Following the prompts, insert the Calibration Cassette into the Sofia Analyzer and close the drawer. The Analyzer performs the Calibration Check automatically with no user input required.

The Sofia Analyzer indicates when the Calibration Check is completed. Select **OK** to return to the Main Menu.

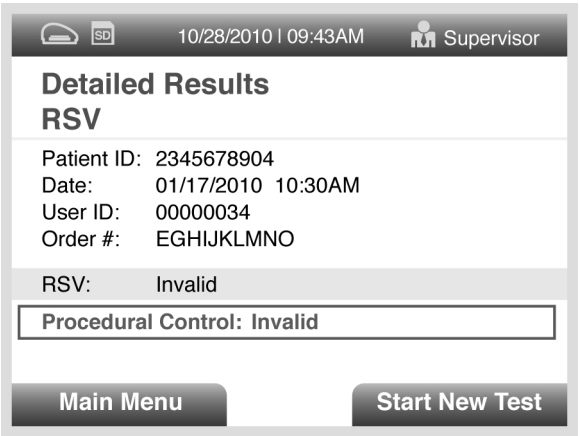
NOTE: If the Calibration Check does not pass, notify the on-site Supervisor or contact Quidel Technical Support for assistance from 7:00 a.m.-5:00 p.m. PST at 800.874.1517 (within the USA); 858.552.1100 (outside the USA); Fax: 858.455.4960; custserv@quidel.com (Customer Service); technicalsupport@quidel.com (Technical Support) or contact your local distributor.

Built-in Procedural Controls

The Sofia RSV FIA contains a built-in procedural control feature. Each time a test is run in the Sofia Analyzer, the procedural control zone is scanned by the Sofia Analyzer and the result is displayed on the Analyzer screen.

The manufacturer's recommendation for daily control is to document the results of these built-in procedural controls for the first sample tested each day. This documentation is automatically logged into the Analyzer with each test result.

A valid result obtained from the procedural control demonstrates that the test flowed correctly and the functional integrity of the Cassette was maintained. **The procedural control is interpreted by the Sofia Analyzer after the Cassette has developed for fifteen (15) minutes. If the test does not flow correctly, the Sofia Analyzer will indicate that the result is invalid.** Should this occur, review the procedure and repeat the test with a new patient sample and a new Cassette.



For example: This display shows an invalid result.

External Quality Control

External Controls may also be used to demonstrate that the reagents and assay procedure perform properly.

Quidel recommends that Positive and Negative External Controls be run:

- once for each untrained operator
- once for each new shipment of kits – provided that each different lot received in the shipment is tested
- as deemed additionally necessary by your internal quality control procedures, and in accordance with local, state and federal regulations or accreditation requirements.

The user must first select Run QC on the Main Menu of the Sofia Analyzer and then, when prompted, scan the QC Card (located on kit box). This card provides information specific to the kit lot, including lot number and expiration date.

The Analyzer will prompt the user to select the desired mode (Walk Away or Read Now) and then to run the External Control swabs.

External Positive and Negative Control swabs are supplied in the kit and should be tested using the Swab Test Procedure provided in this Package Insert or in the Quick Reference Instructions.

When the QC test is complete, each result will be displayed as "Passed" or "Failed" for the Positive Control and the Negative Control.

Do not perform patient tests or report patient test results if either of the QC tests do not produce the expected results. Repeat the test or contact Quidel Technical Support before testing patient specimens, if a "failed" result is obtained with the External Controls.

Additional External Control swabs may be obtained separately by contacting Quidel's Customer Support Services at 800.874.1517 (toll-free in the U.S.A.) or 858.552.1100.

SPECIMEN COLLECTION AND HANDLING

SPECIMEN COLLECTION

Nasopharyngeal Swab Sample

Use the nasopharyngeal swab supplied in the kit.

To collect a nasopharyngeal swab sample, carefully insert the swab into the nostril that presents the most secretion under visual inspection. Keep the swab near the septum floor of the nose while gently pushing the swab into the posterior nasopharynx. Rotate the swab several times then remove it from the nasopharynx.

Nasopharyngeal Aspirate/Wash Sample

Follow your institution's protocol for obtaining nasopharyngeal aspirate/wash specimens. **Use the minimal amount of saline that your procedure allows.** Alternatively, if your institution does not provide a protocol, then consider the following procedures that are used by clinicians.

To collect a nasopharyngeal aspirate sample: instill a few drops of sterile saline into the nostril to be suctioned. Insert the flexible plastic tubing along the nostril floor, parallel to the palate. After entering the nasopharynx, aspirate the secretions while removing the tubing. The procedure should be repeated for the other nostril if inadequate secretions were obtained from the first nostril.

To collect a nasopharyngeal wash sample: the child should sit in the parent's lap facing forward, with the child's head against the parent's chest. Fill the syringe or aspiration bulb with the minimal volume of saline required per the subject's size and age. Instill the saline into one nostril while the head is tilted back. Aspirate the wash specimen back into the syringe or bulb. The aspirated wash sample will likely be approximately 1 cc in volume.

Alternatively, following instillation of the saline, tilt the head forward and let the saline drain out into a clean collection cup.

SPECIMEN TRANSPORT AND STORAGE

Specimens should be tested as soon as possible after collection. However, if transport of samples is required, minimal dilution of the sample is recommended, as dilution may result in decreased test sensitivity. One (1) milliliter or less is suggested for optimal rapid test performance. The following viral transport media listed in Table 1 are compatible with the Sofia RSV FIA:

Table 1
Recommended Viral Transport Media

	Recommended Storage Condition	
Viral Transport Media	**2°C to 8°C**	**25°C**
Copan Universal Transport Media	24 hours	24 hours
Hank's Balanced Salt Solution	24 hours	24 hours
Liquid Amies Media	24 hours	24 hours
M4	24 hours	24 hours
M4-RT	24 hours	24 hours
M6	24 hours	24 hours
Modified Liquid Stuarts Media	24 hours	24 hours
Saline	24 hours	24 hours
Starplex Multitrans	24 hours	24 hours
Phosphate Buffered Saline	24 hours	24 hours

TEST PROCEDURE

All clinical specimens must be at room temperature before beginning the assay.

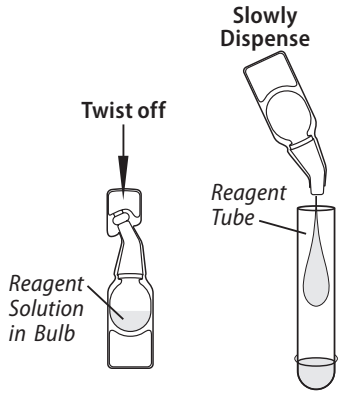
Expiration date: Check expiration date on each individual test package or outer box before using. *Do not use any test past the expiration date on the label.*

Nasopharyngeal Swab Test Procedure

1. Verify that the Sofia Analyzer is set to the desired Analyzer Mode: **Walk Away** or **Read Now.** See the "Using the Sofia Analyzer" section for more information.

2. Prepare Reagent:

- **a.** Flick or shake the Reagent Solution vial down so that all fluid is in the bulb.
- **b.** Twist off the tab.
- **c.** Slowly dispense all of the Reagent Solution into the Reagent Tube.
- **d.** Gently swirl the Reagent Tube to dissolve its contents.



3. Place the patient swab sample into the Reagent Tube. Roll the swab at least three (3) times while pressing the head against the bottom and side of the Reagent Tube.

Leave the swab in the Reagent Tube for one (1) minute.

4. Roll the swab head against the inside of the Reagent Tube as you remove it. Dispose of the used swab in your biohazard waste.

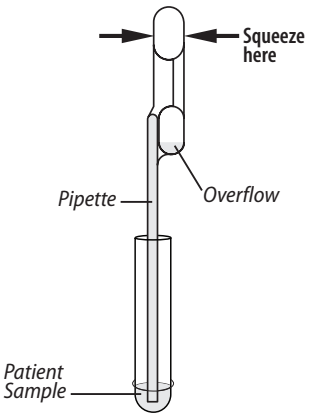
5. Fill the provided **small, clear** fixed volume pipette with the patient sample from the Reagent Tube.

To fill the fixed volume pipette with the patient sample:

a. FIRMLY squeeze the top bulb.

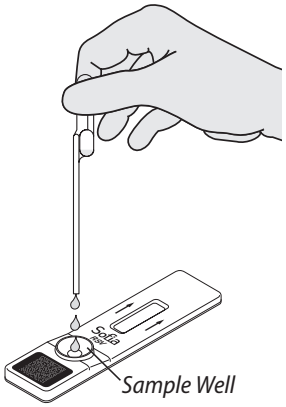
b. Still squeezing, place the pipette tip into the patient sample.

c. With the pipette tip still in the patient sample, release pressure on bulb to fill the pipette.



6. Firmly squeeze the top bulb to empty the contents of the **small, clear** fixed volume pipette into the Cassette sample well. Extra liquid in the overflow bulb is OK.

NOTE: The fixed volume pipette is designed to collect and dispense the correct amount of patient sample. Discard the pipette in your biohazard waste.



7. Proceed to the next section, "Using the Sofia Analyzer," to complete the test.

Nasopharyngeal Aspirate/Wash or Specimens in Viral Transport Media Test Procedure

1. Verify that the Sofia Analyzer is set to the desired Analyzer Mode: **Walk Away** or **Read Now.** See the "Using the Sofia Analyzer" section for more information.

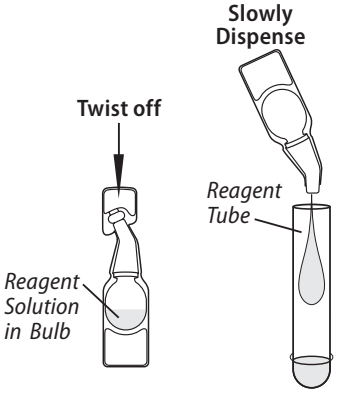
2. Prepare Reagent:

a. Flick or shake the Reagent Solution vial down so that all fluid is in the bulb.

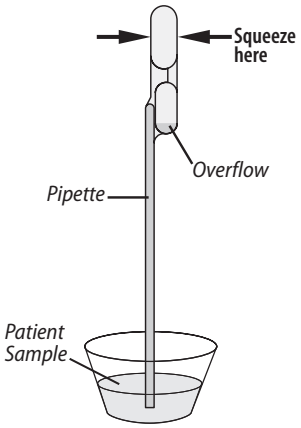
b. Twist off the tab.

c. Slowly dispense all of the Reagent Solution into the Reagent Tube.

d. Gently swirl the Reagent Tube to dissolve its contents.



3. Fill the provided **large, pink** fixed volume pipette with patient sample from the collection cup.



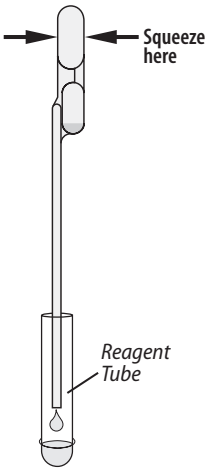
To fill the fixed volume pipette with the sample:

a. FIRMLY squeeze the top bulb.

b. Still squeezing, place the pipette tip into the patient sample.

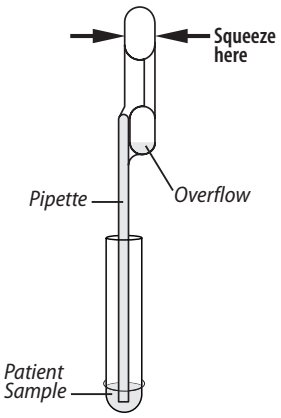
c. With the pipette tip still in the liquid sample, release pressure on bulb to fill the pipette.

4. Firmly squeeze the top bulb to empty the contents of the **large, pink** fixed volume pipette into the Reagent Tube. Extra liquid in the overflow bulb is OK. **Gently swirl the Reagent Tube to mix.**

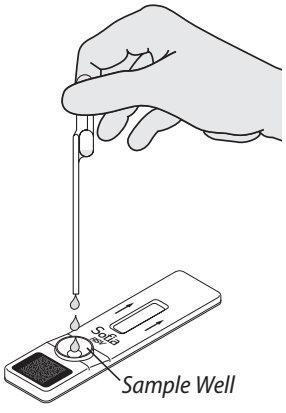


NOTE: The fixed volume pipette is designed to collect and dispense the correct amount of patient sample. Discard the pipette in your biohazard waste.

5. Fill the provided **small, clear** fixed volume pipette with patient sample from the Reagent Tube.



6. Firmly squeeze the top bulb to empty the contents of the **small, clear** fixed volume pipette into the Cassette sample well. Extra liquid in the overflow bulb is OK. Discard the pipette in your biohazard waste.



NOTE: The fixed volume pipette is designed to collect and dispense the correct amount of patient sample. Discard the pipette in your biohazard waste.

7. Proceed to the next section, "Using the Sofia Analyzer," to complete the test.

USING THE SOFIA ANALYZER

Walk Away/Read Now Modes

Refer to the Sofia Analyzer User Manual for operating instructions.

The Analyzer may be set to two different modes (Walk Away and Read Now). The procedures for each mode are described below.

Walk Away Mode

In Walk Away Mode, the user **immediately** inserts the Cassette into the Analyzer. The user then returns after fifteen (15) minutes to get the test result. In this mode, the Analyzer will automatically time the test development before scanning and displaying the test result.

Read Now Mode

Allow the test to develop for the FULL fifteen (15) minutes BEFORE placing it into the Analyzer.

The user must first place the Cassette onto the counter or bench top for fifteen (15) minutes (outside of the Analyzer) and manually time this development step. Less than fifteen (15) minutes may result in false negative results. Then, the user inserts the Cassette into the Analyzer. In Read Now Mode, the Analyzer will scan and display the test result within one (1) minute.
Note: Results will remain stable for an additional fifteen (15) minutes after the recommended development time of fifteen (15) minutes.

Tips for Batch Testing

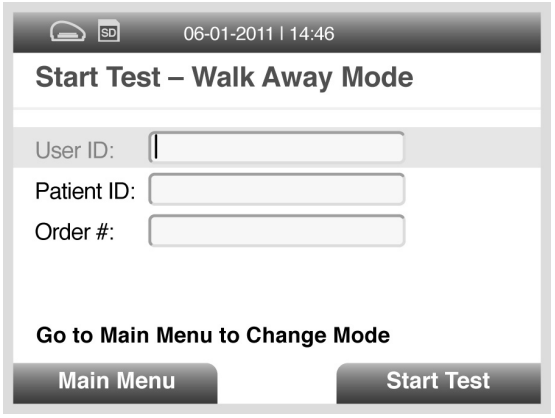
Depending on the workload, several options exist to make batch testing easier. The user can add the Reagent Solution to one or more Reagent Tubes, recap them, and store them on the bench at room temperature for up to 4 hours without loss of activity before adding the sample(s). Alternatively, after addition of the Reagent Solution, the user can process swab or liquid specimens in the Reagent Tube, then after removing the swab (if applicable), recap the tube and let them stand at room temperature for up to 4 hours without loss of activity before testing.

Critically important, the user should never open the foil pouch exposing the Cassette to ambient environment until ready for immediate use.

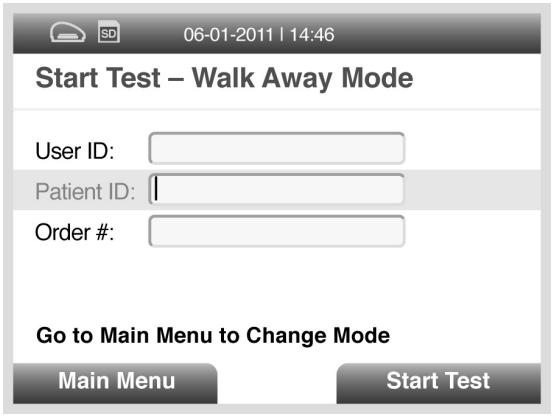
Run Test

1. Input the user ID using the handheld barcode scanner or manually enter the data using the key pad.

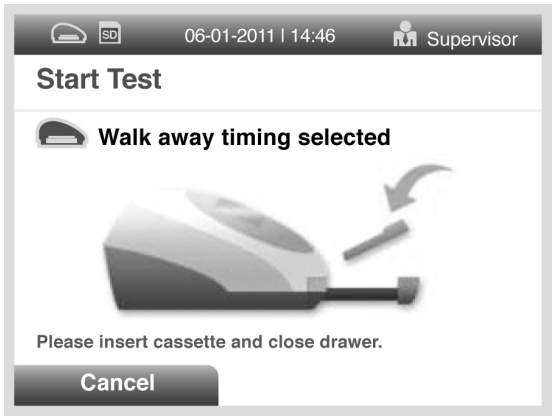
NOTE: *If you mistakenly scan the wrong barcode, use the Arrow Buttons on the Sofia Analyzer key pad to re-highlight the field. Then simply rescan using the correct barcode, and the previous one will be overwritten with the correct barcode.*



2. Input Patient ID or Order # using the handheld barcode scanner or manually enter the data using the key pad.

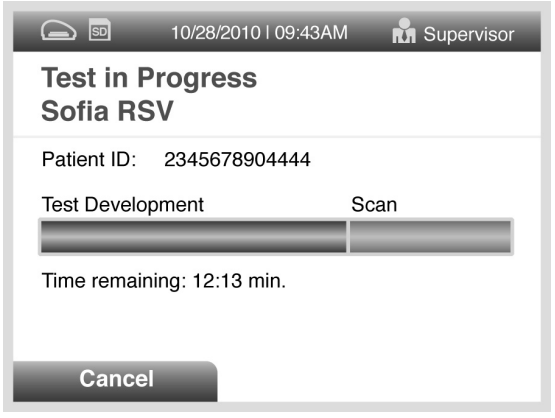


3. Press Start Test and the Sofia Analyzer drawer will automatically open.



4. Verify that the correct development mode, Walk Away or Read Now, has been selected. Insert the prepared patient Cassette into the drawer of the Sofia Analyzer and close the drawer.

5. The Analyzer will start automatically and display the progress, as shown in the example below. In Walk Away Mode, the test results will be displayed on the screen in approximately fifteen (15) minutes. In Read Now Mode, the test results will be displayed on the screen within one (1) minute. See Interpretation of Results section.



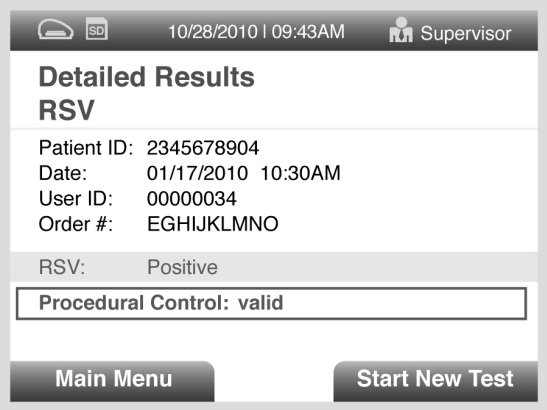
For example: This display shows that the test in Walk Away mode has 12 minutes, 13 seconds remaining. The Sofia Analyzer will read and display the results after 15 minutes.

INTERPRETATION OF RESULTS

When the test is complete, the results will be displayed on the Sofia Analyzer screen. The results can be automatically printed on the integrated printer if this option is selected. Test Lines, which are fluorescent, cannot be seen with the naked eye.

The Sofia Analyzer screen will display results for the procedural control as being "valid or invalid," and will provide a positive or negative result for RSV. If the procedural control is "invalid," retest with a new patient sample and a new Cassette.

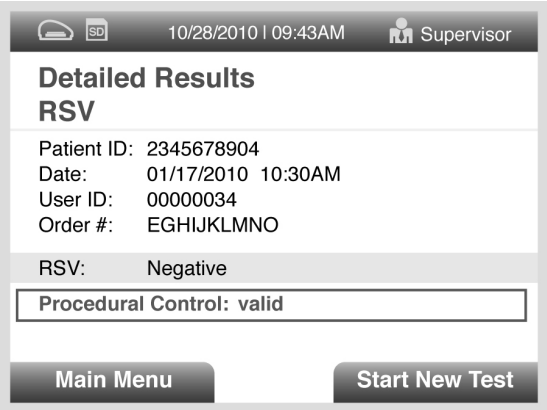
Positive Results:



For example: This display shows a valid <u>positive result for RSV</u>.

NOTE: A positive result does not rule out co-infections with other pathogens.

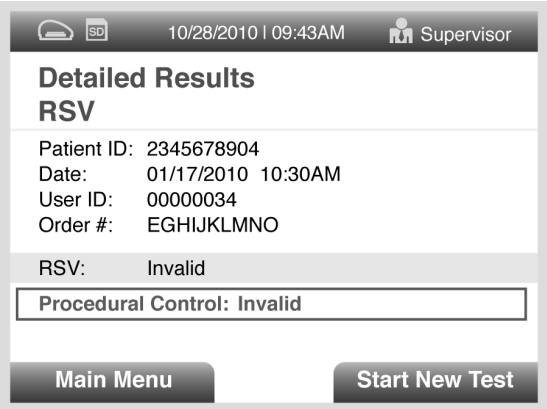
Negative Results:



For example: This display shows a valid <u>negative</u> result for RSV.

NOTE: A negative result does not exclude RSV viral infection. Negative results should be confirmed by viral culture.

Invalid Results:



For example: This display shows an invalid result.

Invalid Result: If the test is invalid, a new test should be performed with a new patient sample and a new Cassette.

LIMITATIONS

- This test is suitable for the pediatric population (less than 19 years of age) only. Performance characteristics have not been established for use with patients older than 19 years of age and for immunocompromised patients.
- The contents of this kit are to be used for the qualitative detection of RSV antigen from nasopharyngeal swab and nasopharyngeal aspirate/wash specimens.
- This test detects both viable (live) and non-viable RSV. Test performance depends on the amount of virus (antigen) in the specimen and may or may not correlate with viral culture results performed on the same specimen.
- A negative test result may occur if the level of antigen in a sample is below the detection limit of the test or if the sample was collected or transported improperly.
- Failure to follow the Test Procedure may adversely affect test performance and/or invalidate the test result.
- Test results must be evaluated in conjunction with other clinical data available to the physician.
- Positive test results do not rule out co-infections with other pathogens.
- Negative test results are not intended to rule in other non-RSV viral or bacterial infections.
- Positive and negative predictive values are highly dependent on prevalence. False negative test results are more likely during peak activity when prevalence of disease is high. False positive test results are more likely during periods of low RSV activity when prevalence is moderate to low.
- Monoclonal antibodies may fail to detect, or detect with less sensitivity, RSV viruses that have undergone minor amino acid changes in the target epitope region.
- Samples contaminated with whole blood >1% may interfere in the interpretation of the test. Visually bloody samples should not be used.
- *Mycoplasma pneumoniae* at levels greater than $1x10^5$ cfu/mL may cross-react or interfere with the performance of the test.
- The performance of this test has not been evaluated for use in patients without signs and symptoms of respiratory infection.

EXPECTED VALUES

The rate of positivity observed in RSV testing will vary depending on the method of specimen collection, handling/transport system employed, detection method utilized, time of year, age of the patient, and disease prevalence. The prevalence observed with culture during the clinical study was 12% (211/1755).

PERFORMANCE CHARACTERISTICS

Sofia RSV FIA Performance vs. Cell Culture

The performance of the Sofia RSV FIA was compared to viral cell culture methods followed by DFA in a multi-center clinical field study during February through April of 2012 and October through December of 2012 in the United States. This study was conducted by health care personnel at 17 distinct sites in various geographical regions within the United States. In this multi-center, point-of-care (POC) field trial, two (2) nasopharyngeal swabs or nasopharyngeal aspirate/wash specimens were collected from each of 1,736 patients. A pair of nasopharyngeal swab specimens was provided by 972 patients and a nasopharyngeal aspirate/wash specimen was provided by 764 patients. All clinical samples were collected from symptomatic patients (less than 19 years of age): 55% were male and 45% were female.

On-site testing of one nasopharyngeal swab specimen or a portion of nasopharyngeal aspirate/wash specimen was performed by medical personnel in the physician's office or hospital facility with the Sofia RSV FIA. The samples were freshly collected and tested. The remaining sample was placed in viral transport media for culturing. The paired swab samples were randomized with respect to the order of testing in the Sofia RSV FIA versus culture. Viral cell culture was performed either at a local clinical laboratory at the test site, or the samples were transported cold on ice packs, not frozen, overnight to a central laboratory for culture within 48 hours. Results are presented in Tables 2 and 3.

Table 2
Sofia RSV FIA Nasopharyngeal Swab Results Versus Culture

	Culture	
	Pos	Neg
Sofia Pos	126	25
Sofia Neg	20	801
Total	146	826

Sens. = 126/146 = 86%
(95% C.I. 80-91%)
Spec.= 801/826 = 97%
(95% C.I. 96-98%)
PPV = 83% (126/151)
NPV = 98% (801/821)

Table 3
Sofia RSV FIA Nasopharyngeal Aspirate/Wash Results Versus Culture

	Culture	
	Pos	Neg
Sofia Pos	57	12
Sofia Neg	7	688
Total	64	700

Sens. = 57/64 = 89%
(95% C.I. 79-95%)
Spec.= 688/700 = 98%
(95% C.I. 97-99%)
PPV = 83% (57/69)
NPV = 99% (688/695)

Sofia RSV FIA Performance vs. Cell Culture When Testing Specimens Placed into Viral Transport Media

The performance of the Sofia RSV FIA when testing specimens placed into VTM was compared to viral cell culture methods followed by DFA in the same multi-center clinical field study during February through April of 2012 and October through December of 2012 in the United States. This portion of the study was conducted by laboratory personnel at two (2) distinct laboratory sites within the United States. A nasopharyngeal swab or nasopharyngeal aspirate/wash specimen collected from each of 1,749 patients was placed in viral transport media and then transported cold on ice packs, not frozen, overnight to the laboratory. The Sofia RSV FIA test was performed on a portion of each specimen, and the culture was performed using the remainder of the same specimen in VTM. Nasopharyngeal swab specimens were provided by 968 patients and nasopharyngeal aspirate/wash specimens were provided by 781 patients. Results are presented in Tables 4 and 5.

Table 4
Sofia RSV FIA Nasopharyngeal Swab in VTM Results Versus Culture

	Culture	
	Pos	Neg
Sofia Pos	125	26
Sofia Neg	18	799
Total	143	825

Sens. = 125/143 = 87%
(95% C.I. 81-92%)
Spec.= 799/825 = 97%
(95% C.I. 95-98%)
PPV = 83% (125/151)
NPV = 98% (799/817)

Table 5
Sofia RSV FIA Nasopharyngeal Aspirate/Wash in VTM Results Versus Culture

	Culture	
	Pos	Neg
Sofia Pos	59	12
Sofia Neg	8	702
Total	67	714

Sens. = 59/67 = 88%
(95% C.I. 78-94%)
Spec.= 702/714 = 98%
(95% C.I. 97-99%)
PPV = 83% (59/71)
NPV = 99% (702/710)

Reproducibility Studies

The reproducibility of the Sofia RSV FIA was evaluated at three (3) different laboratories. Two (2) different operators at each site tested a series of coded, contrived samples, prepared in negative clinical matrix, ranging from low negative to moderate positive RSV. The inter-laboratory agreement (Table 6) for negative samples was 98%-100% and 98%-100% for positive samples. The intra-laboratory agreement (Table 7) for all samples ranged from 98%-100%.

Table 6
Sofia RSV FIA Reproducibility Study Inter-laboratory Agreement

Site	Low Neg (no virus)	High Negative (C_5)	Low Positive (C_{95})	Mod. Positive ($C_{3X\ LoD}$)
1	30/30	28/30	30/30	30/30
2	30/30	30/30	28/30	30/30
3	30/30	30/30	30/30	30/30
Total	90/90	88/90	88/90	90/90
% Overall Agreement (95% CI)	**100% (95%-100%)**	**98% (92%-100%)**	**98% (92%-100%)**	**100% (95%-100%)**

Table 7
Sofia RSV FIA Reproducibility Study Intra-laboratory Agreement

Site	Low Neg (no virus)	High Negative (C_5)	Low Positive (C_{95})	Mod. Positive ($C_{3X\ LoD}$)	% Overall Agreement (95% CI)
1	30/30	28/30	30/30	30/30	**98%** (118/120) **(94%-100%)**
2	30/30	30/30	28/30	30/30	**98%** (118/120) **(94%-100%)**
3	30/30	30/30	30/30	30/30	**100%** (120/120) **(96%-100%)**

Limit of Detection and Analytical Reactivity

The limit of detection (LOD) for the Sofia RSV FIA was determined using a total of four (4) strains of RSV, two (2) isolates of RSV A and two (2) isolates of RSV B (Table 8).

Table 8
Limit of Detection with Human Isolates of RSV A and B

Viral Strain	Minimum Detectable Level ($TCID_{50}$/mL)
RSV A-2	3153
RSV A Long	372
RSV B CH93-18(18)	476
RSV B Washington/18537/62	32.3

$TCID_{50}$/mL=50% tissue culture infectious dose. $TCID_{50}$ levels were determined by the Reed-Muench method.

Analytical reactivity was demonstrated using two (2) additional strains of RSV B: West Virginia Strain/14617/85 at 163 $TCID_{50}$/mL and RSV 9320 at 8.7 $TCID_{50}$/mL.

Analytical Specificity

Cross Reactivity

The cross reactivity of the Sofia RSV FIA was evaluated with a total of 32 bacterial and fungal microorganisms and 42 non-RSV viral isolates. None of the organisms or viruses listed below in Table 9 showed any sign of cross reactivity in the assay. When the same organisms in Table 9 were pre-mixed with RSV and tested in the Sofia RSV FIA, all results were positive indicating that the potential cross-reactants did not interfere with the detection of RSV.

Table 9
Analytical Specificity and Cross Reactivity

Organism/Non-RSV Virus	Concentration*
Acinetobacter baumannii	$2.32x10^6$ cfu/mL
Bacteroides fragilis	$2.32x10^6$ cfu/mL
Bordetella pertussis	$2.32x10^6$ cfu/mL
Candida albicans (yeast)	$2.32x10^6$ cfu/mL
Corynebacterium diptheriae	$2.32x10^6$ cfu/mL
Escherichia coli	$2.32x10^6$ cfu/mL
Haemophilus influenzae	$2.32x10^6$ cfu/mL
Klebsiella pneumoniae	$2.32x10^6$ cfu/mL
Lactobacillus plantarum	$2.32x10^6$ cfu/mL
Legionella pneumophila	$2.32x10^6$ cfu/mL
Moraxella catarrhalis	$2.32x10^6$ cfu/mL
Mycobacterium avium	$2.32x10^6$ cfu/mL
Mycobacterium intracellulare	$2.32x10^6$ cfu/mL
Mycobacterium tuberculosis	$2.32x10^6$ cfu/mL
Mycoplasma pneumoniae	$1x10^5$ cfu/mL
Neisseria meningitides	$2.32x10^6$ cfu/mL
Neisseria mucosa	$2.32x10^6$ cfu/mL
Neisseria sicca	$2.32x10^6$ cfu/mL
Neisseria subflava	$2.32x10^6$ cfu/mL
Pseudomanas aeruginosa	$2.32x10^6$ cfu/mL
Serratia marcescens	$2.32x10^6$ cfu/mL
Staphylococcus aureus	$2.32x10^6$ cfu/mL
Staphylococcus aureus (Cowen 1)	$2.32x10^6$ cfu/mL
Staphylococcus epidermidis	$2.32x10^6$ cfu/mL
Streptococcus mutans	$2.32x10^6$ cfu/mL
Streptococcus pneumoniae	$2.32x10^6$ cfu/mL
Streptococcus pyogenes Group A	$2.32x10^6$ cfu/mL
Streptococcus sanguis	$2.32x10^6$ cfu/mL
Streptococcus sp. Group B	$2.32x10^6$ cfu/mL
Streptococcus sp. Group C	$2.32x10^6$ cfu/mL
Streptococcus sp. Group F	$2.32x10^6$ cfu/mL
Streptococcus sp. Group G	$2.32x10^6$ cfu/mL
Adenovirus 3	$2.32x10^5$ $TCID_{50}$/mL
Adenovirus 4	$2.64x10^4$ $TCID_{50}$/mL
Adenovirus 5	$8.98x10^5$ $TCID_{50}$/mL
Adenovirus 7A	$2.32x10^5$ $TCID_{50}$/mL
Adenovirus 11	$2.32x10^5$ $TCID_{50}$/mL
Coranavirus OC43	$2.32x10^5$ $TCID_{50}$/mL

Organism/Non-RSV Virus	Concentration*
Coranavirus 229E	$2.32x10^5$ $TCID_{50}$/mL
Coxsackievirus B5 (Faulkner)	$2.32x10^5$ $TCID_{50}$/mL
Cytomegalovirus AD-169	$2.32x10^5$ $TCID_{50}$/mL
Cytomegalovirus Towne	$2.32x10^5$ $TCID_{50}$/mL
Echovirus Type 3	$2.32x10^5$ $TCID_{50}$/mL
Herpes Simplex virus 1	$2.32x10^5$ $TCID_{50}$/mL
Herpes Simplex virus 2	$2.32x10^5$ $TCID_{50}$/mL
Human Metapneumovirus A1	$2.32x10^5$ $TCID_{50}$/mL
Human Metapneumovirus A2	$2.32x10^5$ $TCID_{50}$/mL
Human Metapneumovirus B1	$2.32x10^5$ $TCID_{50}$/mL
Human Metapneumovirus B2	$2.32x10^5$ $TCID_{50}$/mL
Influenza A H1N1 (Mexico/4108/2009)	$2.32x10^5$ $TCID_{50}$/mL
Influenza A H1N1 (Denver/1/57)	$2.32x10^5$ $TCID_{50}$/mL
Influenza A H1N1 (FM/1/47)	$2.32x10^5$ $TCID_{50}$/mL
Influenza A H1N1 (New Jersey/8/76)	$2.32x10^5$ $TCID_{50}$/mL
Influenza A H1N1 (PR/8/34)	$2.32x10^5$ $TCID_{50}$/mL
Influenza A H3N2	$2.32x10^5$ $TCID_{50}$/mL
Influenza B Hong Kong	$2.32x10^5$ $TCID_{50}$/mL
Influenza B Panama	$2.32x10^7$ $TCID_{50}$/mL
Influenza C/Taylor/1233/47	$2.32x10^5$ $TCID_{50}$/mL
Measles (Edmonston)	$2.32x10^5$ $TCID_{50}$/mL
Metapneumovirus VR-03-00181 UIHC	$2.32x10^5$ $TCID_{50}$/mL
Mumps (Enders)	$2.32x10^5$ $TCID_{50}$/mL
Parainfluenza virus 1	$2.32x10^5$ $TCID_{50}$/mL
Parainfluenza virus 2	$2.32x10^5$ $TCID_{50}$/mL
Parainfluenza virus 3	$2.32x10^5$ $TCID_{50}$/mL
Parainfluenza virus 4A	$2.32x10^5$ $TCID_{50}$/mL
Parainfluenza virus 4B	$2.32x10^5$ $TCID_{50}$/mL
Rhinovirus Type 1B	$2.32x10^5$ $TCID_{50}$/mL
Rhinovirus Type 2	$2.32x10^5$ $TCID_{50}$/mL
Rhinovirus Type 3	$2.32x10^5$ $TCID_{50}$/mL
Rhinovirus Type 7	$2.32x10^5$ $TCID_{50}$/mL
Rhinovirus Type 15	$2.32x10^5$ $TCID_{50}$/mL
Rhinovirus Type 18	$2.32x10^5$ $TCID_{50}$/mL
Rhinovirus Type 37	$2.32x10^5$ $TCID_{50}$/mL
Varicella Zoster Virus	$3.55x10^4$ $TCID_{50}$/mL

*The levels of bacteria were determined by limiting dilution, bacterial culture, and colony counting to give cfu/mL (cfu=colony forming unit). Virus concentrations were determined by standard virology methods, Reed-Muench.

Interfering Substances

Whole blood, mucin, and several over-the-counter (OTC) products and common chemicals were evaluated and did not interfere with the Sofia RSV FIA at the levels indicated below (Table 10).

Table 10
Non-interfering Substances

Substance [Active Ingredient]	Concentration
OTC Mouthwash #1 (Listerine)	58%
OTC Mouthwash #2 (Crest Pro-Health)	58%
OTC Mouthwash #3 (Scope)	58%
OTC Cough Drop #1 (CVS)	19%
OTC Cough Drop #2 (Ricola)	15%
OTC Cough Drop #3 (Halls)	34%
Nasal Spray #1 (Vick's)	23%
Nasal Spray #2 (4-Way)	23%
Nasal Spray #3 (Equate)	23%
Whole Blood	1%
Acetamidophenol	23 mg/mL
Acetylsalicylic acid	23 mg/mL
Chlorpheniramine	4 mg/mL
Dextromethorphan	4 mg/mL
Diphenhydramine	3 mg/mL
Mucin	9 mg/mL
Guaiacol	46 mg/mL
Phenylephrine	11 mg/mL
Rimantadine	116 µg/mL
Albuterol	26 mg/mL

ASSISTANCE

If you have any questions regarding the use of this product or if you want to report a test system problem, please call Quidel's Technical Support Number 800.874.1517 (toll-free in the U.S.A.) or 858.552.1100, Monday through Friday, between 7:00 a.m. and 5:00 p.m., Pacific Time, U.S.A. If outside the United States contact your local distributor or technicalsupport@quidel.com.

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